Trends of Sporadic Leptospirosis in Florida

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SEROLOGIC EVIDENCE of leptospirosis in human beings and domestic animals in Florida has existed since 1951. Between 1951 and 1954, a total of 1,296 specimens of blood from persons and animals with suspected cases were tested in laboratories of the Florida Division of Health, using agglutination-lysis, complement-fixation, or both techniques. Three of the 162 serum specimens from persons (1.8 percent) yielded positive results; 352 of 847 serum specimens from cattle (41.5 percent) and 136 of 271 serum specimens from dogs (50 percent) tested positive (1). Three more cases of leptospirosis in human beings were diagnosed in the next 2 years. In the latter part of 1957, the laboratories began to screen routinely for leptospirosis all human serum specimens submitted for study because of central nervous system complaints.

Three of the authors are with the Florida Division of Health, Jacksonville: Mr. Bigler is a biologist and Dr. Nichols is the administrator, veterinary public health section; Dr. Prather is the administrator of the epidemiology section, bureau of preventable diseases. Dr. Collins, at the time of the study, was an Epidemic Intelligence Service officer of the National Communicable Disease Center, Public Health Service, on assignment to the Florida Division of Health. He is now associated with the Veterans' Administration Hospital, Cleveland, Ohio. Until her death, Mrs. Galton was chief of the Veterinary Public Health Laboratory, National Communicable Disease Center, Atlanta, Ga. Dr. Richard Skinner and Dr. James Walker, pediatricians, and Dr. James Fish and Dr. Joseph Cooksey, veterinarians, who practice in Jacksonville, assisted in the epidemiologic studies.

Our report is an analysis of 87 cases of leptospirosis that occurred in Florida in human beings during the 10-year period 1958-67; two case studies exemplify *Leptospira canicola* infections in children that were attributed to contact with hunting dogs.

Methods

Serum specimens from persons with an illness referrable to the central nervous system were routinely tested for leptospirosis at the laboratory of the Florida Division of Health in Jacksonville. The rapid macroscopic slide agglutination test was used with pooled bacto-leptospiral antigens. Serum specimens that tested positive in one or more pools were sent to the Veterinary Public Health Laboratory, National Communicable Disease Center, Atlanta, Ga., for microscopic agglutination testing.

Twelve antigens were routinely used: Leptospira ballum, L. canicola, Leptospira icterohaemorrhagiae, Leptospira bataviae, Leptospira grippotyphosa, Leptospira pyrogenes, Leptospira autumnalis, Leptospira pomona, Leptospira seiroe, Leptospira australis, Leptospira tarassovi, and Leptospira mini-georgia. Serums showing titers to L. sejroe were tested also against Leptospira hardjo, Leptospira wolffi, and Leptospira wolffi A antigens. Serum samples with titers to L. autumnalis were tested additionally with the Fort Bragg antigen. In some instances, serum samples that were negative in the initial battery were subsequently tested with Leptospira andamana, Leptospira javanica, and Leptospira semaranga or Leptospira patoc 1 antigens.

Clinical and epidemiologic histories and sero-

logic test results were carefully evaluated in each case. Those persons who had a clinical history compatible with a recent infection, an epidemiologic history suggesting they were infected in Florida, and positive results in macroscopic or microscopic agglutination tests were considered to have leptospirosis.

In the summer of 1967, a group of Jacksonville pediatricians detected *L. canicola* infections in two 12-year-old boys who routinely cared for the family hunting dogs. After preliminary inquiries revealed that the families of the two boys were acquainted, the boys and their mothers were interviewed by a veterinarian and a medical epidemiologist. At each residence, blood specimens were collected from all dogs on the premises, and urine was obtained from selected dogs by bladder catheterization.

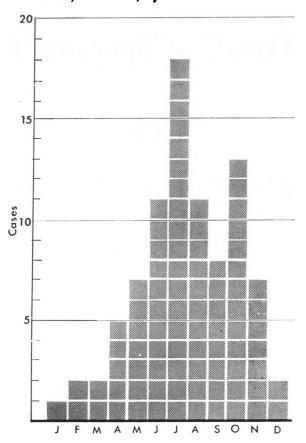
All urine samples were diluted with buffered saline to final dilutions of 10⁻¹, 10⁻², and 10⁻³, then inoculated into Fletcher's semisolid medium with 10 percent rabbit serum. Wild mammals were trapped on and around the premises, anesthetized with chloroform, and exsanguinated by intracardiac puncture. At necropsy, 0.1 ml. of urine was obtained by bladder puncture, serially diluted to 10⁻¹ and 10⁻² with a buffered saline solution, and inoculated into Fletcher's medium. Portions of both kidneys were aseptically removed and ground in a mortar with sterile alundum suspended in a buffered saline solution. Approximately 0.1 ml. of 10-2 and 10-3 dilutions were also inoculated into the medium.

Inoculated tubes were shipped to the Veterinary Public Health Laboratory, National Communicable Disease Center, for incubation and periodic microscopic examination. The samples of animal serum were tested for *Leptospira* agglutinins by the methods described. A review of each patient's hospitalization records and followup home visits completed the epidemiologic studies.

Results

Over the 10-year period 1958-67, a total of 6,066 persons throughout the State were screened for leptospirosis by macroscopic slide agglutination tests; 86 were considered to have probable cases of leptospirosis. Serologically,

Figure 1. Distribution of the 87 cases of leptospirosis reported in human beings in Florida, 1958-67, by month



these persons could be divided into four categories according to whether there was: (a) a fourfold rise or fall in titers by microscopic testing in paired serums-53 cases, 61 percent; (b) any change in microscopic titer-15 cases, 17 percent; (c) a constant high microscopic titer of 1:100 or more—11 cases, 13 percent; or (d) a high macroscopic titer (definite clumping of leptospires in at least one pool of antigen when the serum was serially diluted in single or paired serum specimens)—7 cases, 8 percent. In one additional case, serum specimens from the patient were not tested; leptospires, however, were demonstrated after death in kidney sections stained by Dieterle's method and in the peritoneal fluid of a hamster which was inoculated with ground liver obtained at autopsy.

The distribution of the 87 cases by month of onset is shown in figure 1. Sixty-two (71 percent) had their onset during the 5-month period

of June through October. The cases by age and sex (known in 74 instances) are shown in figure 2. Thirty-nine (52.7 percent) occurred in persons less than 20 years of age; the highest number of cases, 24 (32.4 percent), were in the 10 to 19-year-old group.

Serologic data from microscopic tests showed that the presumed infective agents in 78 of the 86 cases belonged to the following 10 serogroups:

Presumed infective serotype	Number of cases	Percent of cases	
L. canicola	36	46. 1	
L. pomona	13	16. 6	
L. icterohaemorrhagiae L. icterohaemorrhagiae or L.	9	11. 5	
canicola	5	6. 4	
L. autumnalis (Fort Bragg)	4	5. 1	
L. grippotyphosa	$ar{2}$	2. 6	
L. tarassovi	$\overline{f 2}$	2. 6	
L. patoc 1	f 2	2. 6	
Hebdomadis serogroup	$ar{f 2}$	2. 6	
L. semaranga	1	1. 3	
L. bataviae	1	1. 3	
L. javanica	1	1. 3	
Total	78	100. 0	

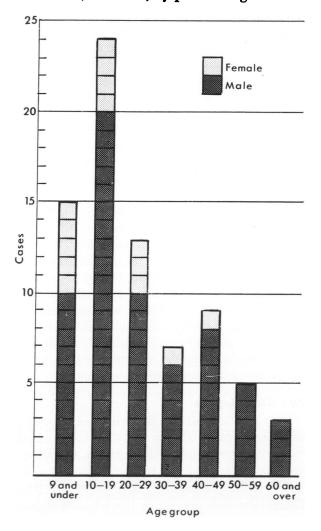
Infective serotypes were reasonably well established in most cases even though some serums showed varying degrees of cross-agglutination with other Leptospira antigens. Five cases in which titers were equally high against the L. canicola and L. icterohaemorrhagiae antigens were classified as L. icterohaemorrhagiae or L. canicola. The two cases of L. patoc 1 are listed separately from the cases of L. semaranga even though they are in the L. semaranga serogroup; the serum of the patient whose infection was attributed to L. semaranga was not tested with the L. patoc 1 antigen.

As could be expected, most of the patients with cases attributed to *L. canicola* and *L. icterohaemorrhagiae* had a history of contact with dogs or rats; the cases attributed to *L. pomona* were usually related to contact with cattle and swine. The only recorded epidemic of human leptospirosis in Florida was attributed to *L. pomona*. In this 1958 outbreak, nine children in Madison County became ill after swimming in a stream that coursed through pastures known to serve infected swine and cattle (2).

Two of the four patients with antibodies against L. autumnalis (Fort Bragg) reported

they had been in contact with water before becoming ill. Two dairy workers on separate farms showed antibodies against several serotypes in the hebdomadis serogroup. In each instance the owners of the dairy farm involved had purchased additional animals a short time before the worker became ill. One of the patients showing antibodies against *L. tarassovi* was a logger who swam in lakes and drank from clear streams while working in the woods. The cases attributed to *L. bataviae* and *L. javanica* are notable because the patients were apparently infected in Florida. In both cases, the specific source of infection appears to have been contact with water. Unfortunately, the source of infection was not

Figure 2. Distribution of 74 cases of leptospirosis reported in human beings in Florida, 1958-67, by patient's age and sex



determined in either of the cases attributed to L. grippotyphosa.

The cases attributed to *L. patoc 1* and to *L. semaranga* deserve special mention since many investigators believe these "biflexa complex" strains of *Leptospira* are nonpathogenic. Both of the patients with illness caused by *L. patoc 1* complained of headache, fever, vomiting, and chills; in addition, one mentioned joint and abdominal pain, while the other exhibited jaundice, nuchal rigidity, and anuria.

Three serum specimens collected from the first patient within 22 days showed a rise in titer for L. patoc 1 from 1:50 to 1:200 with a corresponding rise from no reactivity to a titer of 1:100 for L. ballum, L. canicola, and L. icterohaemorrhagiae. Serum samples drawn at the time of the second patient's admission to the hospital and 3 days later during a post mortem examination showed a rise from no reactivity to a titer of 1:100 for L. patoc 1 and revealed no cross reactivity with other serotypes.

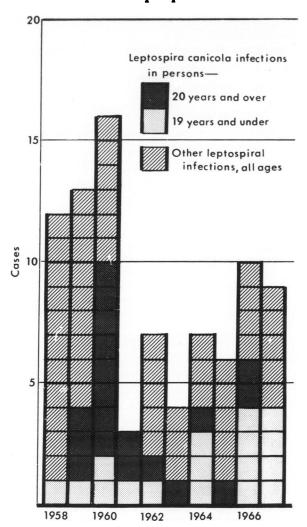
The patient with the infection caused by L. semaranga, whose case was diagnosed as aseptic meningitis, had a fever and nuchal rigidity. Three serum specimens drawn at monthly intervals showed microscopic titers to L. semaranga of 1:3,200 in S_1 and S_2 and 1:1,600 in S_3 . The third serum specimen showed reactivity also for L. and amana (titer of 1:50), Leptospira djasmin (titer of 1:200), and L. canicola (titer of 1:400).

Since 1958, L. canicola has been the probable infecting serotype in almost half of Florida's identified human cases of leptospirosis (fig. 3.). These infections have been generally distributed throughout the age spectrum, but between 1964 and 1967, 11 of the 15 cases attributed to this serotype occurred in children between 6 and 12 years of age. Seeking epidemiologic definition of the various sources and transmission patterns of L. canicola infections, we undertook an intensive investigation of two selected cases.

Two Case Reports

Case 1. A 12-year-old boy who had been well until the morning of May 6, 1967, awoke on that date with fever, nausea, and myalgia. On physical examination he had a temperature of 99.4° F. and a blood pressure of 100 over 48 mm. Hg. Tenderness was present in the left costoverte-

Figure 3. Distribution of the 87 cases of leptospirosis reported in human beings in Florida, 1958-67, with age groups of the 36 infected with Leptospira canicola



bral angle. Other results of the examination were within the normal range, as were the results of routine urinalysis.

Late the following day, because of abdominal pain, elevated temperature, and vomiting, the boy was admitted to a local children's hospital. On admission he appeared weak and moderately dehydrated. Marked tenderness in the left costovertebral angle persisted, and some tenderness was present in the right lower quadrant. His abdomen was soft, without guard, rebound tenderness, or distention. The spleen was felt 2 cm. below the left costal margin. The results of examination of the chest and abdomen were nega-

tive. There were 12.1 gm. of hemoglobin per 100 cc. of blood, the hematocrit value was 35 percent, and the white cell count per cubic millimeter of blood was 5,000. The urine was normal, and a Monotest gave negative results. The results of agglutination tests for salmonellae and brucellae were negative, and the Weil-Felix reaction was also negative. The boy was given acetylsalicylic acid for temperature control, and his abdominal pain ceased following an enema. The tenderness observed in the costovertebral angle subsided, and the patient was discharged on May 9.

Three days later, May 12, the boy returned to the pediatrician's office with fever, headache, and neck pain. He had a temperature of 100° F. and exhibited nuchal rigidity. He was readmitted to the hospital the same day. A physical examination revealed no abnormalities other than a stiff neck.

The hemoglobin was 12.1 gm., the hematocrit value 35 percent, and the white cell count 7,500; 7 percent of the leukocytes were band forms, 72 percent polymorphonuclear granulocytes, 17 percent lymphocytes, and 4 percent monocytes. The urine was normal. Lumbar puncture revealed slightly cloudy cerebrospinal fluid under

normal pressure Microscopic examination of the fluid showed 140 white cells per cubic millimeter; 40 percent of the leukocytes were polymorphonuclear granulocytes and 60 percent lymphocytes. The protein content was 65 mg., and the glucose content, 30 mg. per 100 cc. No organisms were present on gram stain, and a bacterial culture yielded no growth.

The boy's headache, fever, and nuchal rigidity abated the day following his admission. He was discharged on May 16. Blood specimens obtained on May 12 and May 23 were forwarded to the laboratory of the Florida Division of Health. A positive result in the macroagglutination test for leptospirosis was reported May 29. Microscopic agglutination showed a *L. canicola* titer of 1:50 in S₁ and 1:400 in S₂ (see table).

The pediatrician noted in retrospect that the patient had been depressed while he was in the hospital because one of his dogs had died. This boy lived in a brick home, on a well-kept lot, in a typical surburban area. A swimming pool was located in the backyard. A kennel and other outbuildings in various stages of disrepair occupied an adjoining lot. Evidence of an infestation by rats was obvious along a concrete

Results of microscopic agglutination tests for leptospirosis in two case studies, Florida, 1967

Date in 1967 serum specimen	Reciprocal of agglutinin titer against antigen for leptospiral serotype				
was collected		ballum	canicola	ictero- haemorrhagiae	pyrogenes
Case 1					
Patient	May 12		50 400	200	50
Kennel dogs:	(y				
v-48	August 9				200
	(August 9				100
v-49 1					100
	October 12				100
	October 25				100
v-50	August 9	100			
Norway rat, 67L-12	August 22		100		
Case 2					
Patient	(July 24		100		- <i></i>
1 440414	July 24				
Kennel dogs:	(8				
v-38	August 9				200
v-41	August 9				
House dog, v-35	August 9	200	800		400

¹ Leptospira canicola isolated from the urine on September 22.

² Leptospira canicola isolated from the kidneys on September 11.

fence, around the foundations of the outbuildings, and in piles of wood, shingles, and other debris. The kennel consisted of 1½-inch diameter metal pipes set into a concrete slab; the top, sides, and door were covered with a large mesh wire. The dogs were sheltered in a series of small open sheds within the wire enclosure. During the investigation several Norway rats (Rattus norvegicus) were observed entering the kennel to feed on scraps of dried food.

On August 9, blood samples were collected from four hunting dogs housed in the kennels, two yard dogs, and one house dog. Serum specimens from the kennel dogs showed positive results in the macroscopic agglutination test. The specimens had titers ranging from 1:400 to 1:800 against L. canicola in the microscopic test (see table). On August 29, the seven dogs underwent bladder catheterization at a local veterinary hospital. Diluted urine samples were inoculated into Fletcher's medium and incubated at 30° C. Twenty-five days after inoculation, L. canicola organisms were isolated from the urine of one of the kennel dogs whose serologic results were positive. Kidney cultures from one of the four Norway rats which had been trapped adjacent to the kennel in early August vielded L. canicola organisms 20 days after inoculation.

Case 2. A 12-year-old boy who had been in good health until July 15 experienced a headache on that date. The following day his temperature rose to 102° F. and anorexia occurred. The fever continued the next day; the boy complained of epigastric pain, nausea, and vomiting. His white cell count was 8,300 per cubic centimeter of blood; the proportion of lymphocytes in the differential count was 36 percent, and the morphology of some of the leukocytes was possibly atypical. Urinalysis showed protein, grade 3+, and 8 to 10 white blood cells per high-powered microscopic field. Chloramphenicol, in 250 mg. doses, four times daily, was started.

When the patient was referred to a pediatrician on July 19, he appeared in good health. He had a temperature of 99.6° F. and a blood pressure of 100 over 62 mm. Hg. The results of a physical examination were unremarkable, and the etiology of his febrile illness was unknown.

Urinalysis at this time did not show protein. The following day the boy was asymptomatic, and his temperature was 98.6° F. On July 21, the mother of the boy in case 1 telephoned the mother of the boy in case 2 and suggested that their sons had the same illness. The two families were acquainted through membership in one church. The mother of the first boy learned of the second boy's illness through mutual friends.

The following day, July 22, the second patient complained of a severe headache and had a temperature of 100° F. His neck was supple, and the remainder of his physical examination revealed no abnormalities. Blood specimens for leptospiral serology were drawn on July 24 and August 2. Both were positive for leptospirosis in the macroagglutination test. Live antigens in the microscopic tests showed a titer for L canicola of 1:100 in S_1 and 1:400 in S_2 (see table). The boy subsequently remained well.

The second patient lived on a small 10-acre farm in an area of slash-pine flatwoods, most of which had been cleared and was used for homes. The farm consisted of pasture, various buildings, a large hog pen, and a 1/4-acre pond, in which the patient swam. This manmade pond was fed by rainwater and an artesian well. It was surrounded by an embankment which prevented water from the hog pen and surrounding pastures from draining into it. The dog kennel was constructed of pipe and wire on a concrete base and consisted of three separate compartments, with three dogs in each. There was very little debris in the area, but the feed room in a nearby storage building supported a moderate population of Norway rats. No rats were seen in or near the kennels.

Blood specimens were not collected during the investigation from the cows, a riding horse, and the fattening swine on the premises. On August 9, blood samples were collected, however, from the nine hunting dogs in the kennel, four yard dogs, and one house dog. Serum specimens from two kennel dogs and the house dog showed titers to *L. canicola* in the microscopic test (see table). Urine samples obtained from several of these dogs failed to yield leptospires within 60 days. Microscopic tests of serum specimens from five Norway rats, four opossums, and one raccoon trapped on August 31

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gave negative results in microscopic tests. No leptospires were isolated from the bladder urine or the kidneys of these animals.

Discussion

Epidemiologic data available on 793 cases of human leptospirosis in the United States between 1947 and 1967 (3-6) show the ages of 628 of the patients. Of this number, 135 (21.4 percent) were 30-39 years of age, and 141 (22.4 percent) were under 20. The infective serotype was reasonably established in 661 cases; 232 cases (35.1 percent) were due to L. icterohaemorrhagiae, 188 (28.4 percent) to L. canicola, and 134 (20.2 percent) to L. pomona. The Florida experience differs from that in the remainder of the country in both the serotypes of Leptospira which were prominent and in the age groups most affected. Among the cases reported in Florida over the 10-year period, seven patients (9.5 percent) were in the 30-39 year age group and 39 (52.7 percent) were in the age group under 20. L. canicola was responsible for 36 cases (46.1 percent), L. pomona for 13 (16.6 percent), and L. icterohaemorrhagiae for 9 (11.5 percent). Seventeen (48.5 percent) of the patients infected with L. canicola were less than 20 years of age. Florida's tropical and subtropical climate and out-of-doors recreation year-round might be expected to increase opportunities for exposure; yet the seasonal distribution of leptospirosis in the State is in accord with that revealed in other studies throughout the nation.

The prevalence of L. canicola infections in Florida children is related to the number of shedders of leptospires in the canine population. During the 6 years 1962-67, the 1,798 cases of canine leptospirosis reported in the State represented 83 percent of all cases reported in animals (2,167). In the United States over the same period, 151,172 cases of animal leptospirosis were reported, but only 46,873-31 percent—were in dogs (3-6). Animals that chronically shed leptospires without displaying any clinical illness are sources of human infection. In 1960, White and associates examined specimens of serum and urine from 193 stray dogs collected in a Florida metropolitan area; almost 33 percent of the 46 animals with positive

serologic test results were shedding leptospires in their urine (7). All evidence suggests that children who come into contact with dogs shedding leptospires run a high risk of leptospiral infection; yet studies of the shedders and associated family members show low infectivity rates (8-10).

The two cases we have reported are examples of infections children contracted as a result of the care and feeding of hunting dogs. During the summer, the boys in the two cases routinely performed these chores barefooted. Since Hubbert and Shotts have shown that penned huntings dogs are capable of shedding L. canicola organisms without having clinical signs of illness (8), we can reasonably assume that the dogs in the kennel and the boys were frequently exposed to infectious urine. Moreover, the fathers of the boys frequently hunted together, and on such occasions, dogs from both kennels shared a common cage on the back of a pickup truck. The last hunting trip occurred 5 or 6 months before the boy in case 1 became ill. The recovery of L. canicola organisms from the kidneys of a Norway rat has been reported from other countries, but to our knowledge ours is the first report of such a natural occurrence in the United States.

Human leptospiral infections give rise to a variety of clinical manifestations. Since 1957, the staff of the bureau of laboratories of the Florida Division of Health have considered all cases of aseptic meningitis and other diseases related to the central nervous system as potential cases of leptospirosis (11). Yet the number of cases of leptospirosis detected by such surveillance tends to be lew because such cases represent only those infections associated with acute febrile illness of the central nervous system. There is no way to determine the number of "mild" leptospiral infections, particularly in adults, that go unrecognized.

In recent years, concerned physicians have been submitting an increasing number of serum specimens from children to the State laboratories, requesting routine viral serologic tests for mumps, measles, and other diseases. Inasmuch as this trend in diagnostic service encourages the examination of more serum specimens from children than from adults, the high proportion of *L. canicola* infections in our under-20 age group may reflect a skewed sample. Without extensive surveys on a scale not so far attempted in Florida, there is no practical way to define the expected levels of leptospiral infection in various age groups.

The epizootology of leptospirosis in Florida, as in other areas, is still unclear. While emphasizing the incidence of *L. canicola* infections in humans and dogs, we cannot ignore the presence of other infective serotypes in dogs, domestic livestock, and wild animals.

Summary

Since 1957, the staff of the Florida Division of Health's bureau of laboratories have considered all cases of aseptic meningitis and other diseases related to the central nervous system as potential cases of leptospirosis. Between 1958 and 1967, an examination of 6,066 serum specimens revealed 86 cases of leptospirosis. In the microscopic agglutination test, 78 of these specimens showed significant agglutinins to the leptospiral organisms of 10 serogroups.

The Florida experience differs from the national one both in the predominant infecting serotype and the age group of patients. In Florida, 46 percent of the leptospirosis cases were attributed to *Leptospira canicola*, while only 28.4 percent of the cases in the United States between 1947 and 1967 were attributed to this serotype. Among the Florida cases, 9.5 percent occurred in persons aged 30–39 years and 52.7 percent in the age group under 20, while national data showed 21.4 percent to be in the 30–39 age group and only 22.4 percent in the group under 20. Between 1964 and 1967, 11 of 15 *L. canicola* cases in Florida occurred in children 6 to 12 years old.

Intensive epidemiologic investigations were conducted of two cases in 12-year-old boys from different families who had cared for the family hunting dogs. L. canicola infections related to contacts with dogs represent the predominant kind of human leptospirosis in Florida.

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Tearsheet Requests

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